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Demographic changes in *Daphnia pulex* (leydig) after exposure to the insecticides spinosad and diazinon

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Abstract

The toxicity of the natural insecticide spinosad was assessed against *Daphnia pulex* (Leydig) using a demographic approach. Data were also generated for the commonly used organophosphorus insecticide diazinon as a comparison. Exposure to spinosad led to a concentration-dependent decline in survival, birth rate (b), net reproductive rate (R_0), and intrinsic rate of increase (r_m). Population extinction ($-r_m$) occurred after exposure to spinosad concentrations $>10\,\mu g/L$ for 8 days. Exposure to increasing diazinon concentrations led to an initial increase in R_0 and r_m followed by a sharp decline, with extinction occurring after exposure to $>2\,\mu g/L$ after 2 days. Based on concentrations of pesticide that caused population extinction, spinosad was five times less toxic than diazinon. The stable age distribution (after 65 days) of *D. pulex* changed after exposure to spinosad and diazinon. Increasing concentrations of spinosad resulted in a decrease in the percentages of individuals in the first juvenile and adult stages, increase in the third and fourth juvenile stages, and little or no change in the second juvenile and adolescent stages. Diazinon had a different effect on stable age distribution. Increasing concentrations of diazinon resulted in an increase in percentages of individuals in the first and second juvenile stages, little or no change in the third and fourth juvenile stages and adolescent stage, and a decrease in the adult stage. Although spinosad and diazinon are both neurotoxins, they have different modes of action and populations of *D. pulex* reacted differently to each pesticide. Results of this study indicate that spinosad is significantly less toxic than diazinon to *D. pulex* and because it is applied at lower concentrations than diazinon it should be less hazardous to this species.

Keywords: Spinosad; Diazinon; *Daphnia pulex*; Demography; Population; Ecotoxicology

1. Introduction

In response to the negative image of pesticides, the pesticide industry has invested extensive time and money into the development of biorational or environmentally benign pesticides. Some of these new pesticides exhibit selective toxicity and thus are more toxic to pest species than to biological control organisms.

One of these new pesticides is spinosad (DowElanco, Indianapolis, IN, USA) (Sparks et al., 1998; Thompson et al., 2000). Spinosad is a mixture of spinosyns A and D, fermentation products of the soil bacterium (*Saccharopolyspora spinosa*, Actinomycetes) (Crouse et al., 2001). Spinosad is a neurotoxin and acts as a contact and stomach poison (DowElanco, 1996; Salgado, 1998; Salgado et al., 1998) and has been shown to be an effective pest control agent (Peck and McQuate, 2000; Brickle et al., 2001) particularly for control of lepidopteran pest species (Wanner et al., 2000). Spinosad is generally more toxic to pest than beneficial insects (Boyd and Boethel, 1998; Pietrantonio and Benedict, 1999; Torres et al., 1999; Elzen and Elzen, 1999; Elzen et al., 1998, 1999, 2000; Elzen, 2001) and is therefore considered a selective insecticide (Miles and Dutton, 2000). However, some studies indicate that spinosad is toxic to beneficial species (Nasreen et al., 2000; Tillman and Mulrooney, 2000; Consoli et al., 2001).

Little is known about the potential effect that spinosad might have on aquatic ecosystems. However, in a previous study, Stark and Banks (2001) examined the effects of several insecticides including spinosad on *Daphnia pulex* by developing acute mortality estimates

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and a population growth rate measure, the instantaneous rate of increase. In the study presented here, we examine in more detail the effects of spinosad on populations of *D. pulex*.

The objective of this study was to evaluate the toxicity of spinosad to a common cladoceran, *D. pulex* (Leydig). A population-level approach was used because previous work has indicated that a better estimate of toxic effect is gained with this method than with traditional toxicological studies using individuals (Forbes and Calow, 1999). The insecticide diazinon, one of the most commonly used insecticides in the world, and the most commonly found insecticide in surface waters in the United States (USGS, 2000), was also evaluated as a standard for comparison.

2. Materials and methods

2.1. Test organisms

D. pulex were obtained from cultures maintained at the Washington State University Research and Extension Center for the past 5 years. Cultures were maintained in a series of 30-mL plastic cups containing 25 mL reconstituted dilution water (RDW) and fed a food solution consisting of a 1:1 mixture of a yeast:cereal leaves:trout chow (YCT) solution and the green alga species *Selenestrum capricornutum*. RDW was prepared by the addition of 1.20 g $MgSO_4$, 1.92 g $NaHCO_3$, 0.080 g KCl, and 1.20 g $CaSO_4 \cdot 2H_2O$ to 20 L distilled, deionized water and subjected to continual aeration, resulting in a RDW with pH 7.4–7.8, conductivity 260–320 μS, dissolved oxygen (DO) >8.0 mg/L, alkalinity of 60–70 mg/L, and hardness 80–100 mg/L. This synthetic fresh water corresponds to a classification of "moderately hard." The YCT solution and RDW were prepared according to the procedures outlined in the US Environmental Protection Agency (USEPA) protocol (1989). A sterile culture of *S. capricornutum* was originally purchased from Charles River Company (Wilmington, MA, USA), and reared on-site using a method modified from USEPA (1989). Neonates were removed daily and transferred to new cups containing RDW and 0.3 mL food solution. Cultures were maintained in an environmental chamber set at 25±0.1°C, 50±5% relative humidity, and 16:8 h light:dark regimen.

2.2. Chemicals tested

Formulated spinosad (Success, 240 gai/L) was obtained from DowElanco. Diazinon Ag 500 was obtained from Novartis, Greensboro, NC, USA.

2.3. Development of life tables

All test organisms were obtained from cultures at or beyond the third filial (F_3) generation. Nominal pesticide concentrations were prepared by serial dilution from a freshly prepared stock solution by the addition of a measured pesticide sample in a defined volume of water. Each test consisted of a minimum of five log-spaced concentrations and a water control. For each nominal concentration tested, 25 mL of test solution was transferred into a 30-mL plastic cup and one neonate was transferred into the test container using a disposable glass pipet. Thirty individuals were tested for each concentration. Test containers were held in an environmental chamber at 25°C.

Survival and reproduction were measured daily (every 24 h) until all animals had died. Test organisms were moved to newly made pesticide solutions every other day (Walthall and Stark, 1997).

Life tables for each insecticide were developed following the approach of Carey (1993). The demographic parameters determined in this study were the intrinsic birth rate (b: the per capita instantaneous rate of birth in the stable population), the net reproductive rate (R_0: the per generation contribution of newborn females to the next generation), the intrinsic rate of increase (r_m: the rate of natural increase in a closed population), and the stable age distribution (the proportion of each age class in a stable population).

3. Results

Exposure to spinosad and diazinon led to a concentration-dependent decline in survival (Fig. 1). However, the response to each insecticide was different. The response to diazinon was typical of organophosphates. Exposure to diazinon resulted in little effect on survival until a threshold was reached (0.9 μg/L) whereby exposure to 0.9 μg/L or more resulted in a high level of mortality within the first 30 days (Fig. 1). The narrow threshold observed with diazinon was not seen with exposure to spinosad. Increasing concentrations of spinosad led to a more gradual decline in survival over a wider range of concentrations (Fig. 1).

The effect of diazinon and spinosad on *D. pulex* birth rate was also different (Fig. 2). Exposure to low concentrations of diazinon actually resulted in an increase in birth rate followed by a rapid decline starting at a concentration of 1.5 μg/L. A concentration of 2 μg/L diazinon completely eliminated birth. Unlike diazinon, a gradual decline in birth rate was observed in *D. pulex* after exposure to increasing spinosad concentrations (Fig. 2). Concentrations of spinosad greater than 10 μg/L completely eliminated birth.

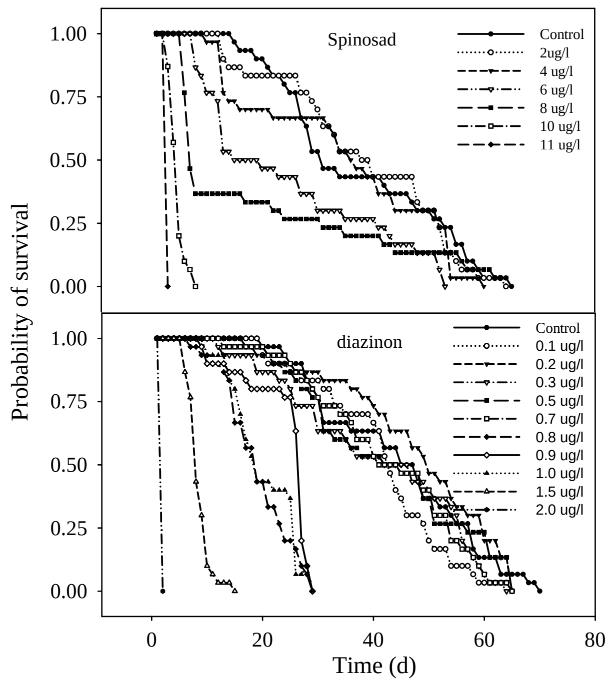

Fig. 1. Survival of *D. pulex* exposed to spinosad and diazinon.

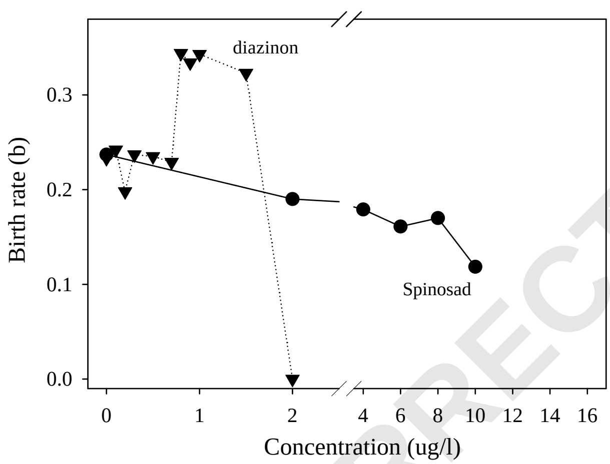

Fig. 2. Birth rate of *D. pulex* exposed to spinosad and diazinon.

A concentration-dependent decline in the net reproductive rate (R_0), and intrinsic rate of increase (r_m) occurred in *D. pulex* with population extinction ($-r_m$) occurring after exposure to spinosad concentrations $>10\,\mu g/L$ for 8 days (Figs. 3 and 4). Exposure to increasing diazinon concentrations led to an initial increase in R_0 and r_m followed by a sharp decline, with extinction occurring after exposure to $>2\,\mu g/L$ for 2 days (Figs. 3 and 4). Based on extinction, diazinon was five times more toxic to *D. pulex* than spinosad. Because R_0 is a measure of reproduction per surviving female, the decline in R_0 caused by spinosad and diazinon indicated that both insecticides affected reproduction. Thus, spinosad and diazinon caused both lethal and sublethal effects in *D. pulex*.

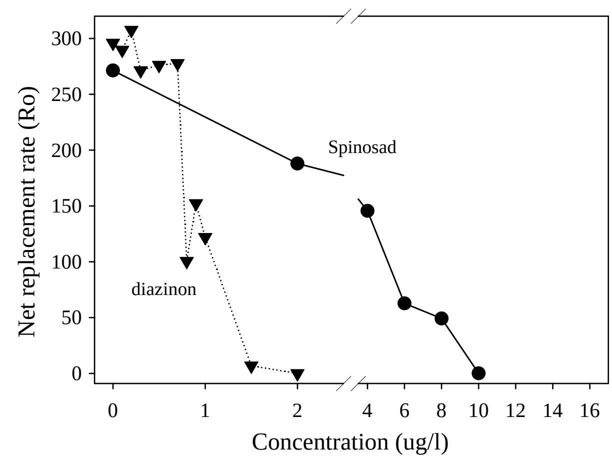

Fig. 3. Net reproductive rate of *D. pulex* exposed to spinosad and diazinon.

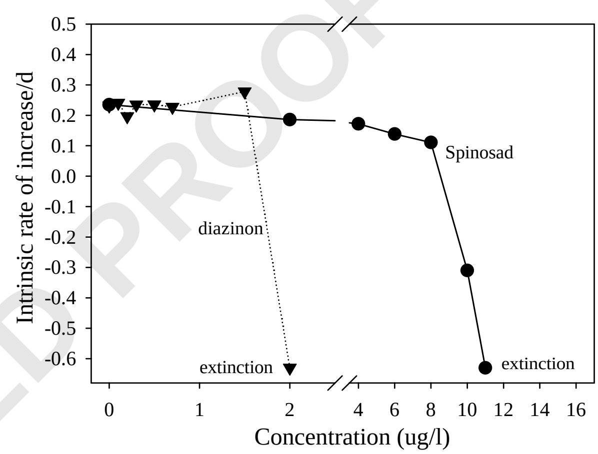

Fig. 4. Intrinsic rate of increase of *D. pulex* exposed to spinosad and diazinon.

The stable age distribution of *D. pulex* changed after exposure to spinosad and diazinon (Table 1). Increasing concentrations of spinosad resulted in a decrease in the percentages of individuals in the first juvenile and adult stages, increase in the third and fourth juvenile stages, and little or no change in the second juvenile and adolescent stages. Exposure to diazinon resulted in a different effect on the stable age distribution (Table 1). Increasing concentrations of diazinon resulted in an increase in percentages of individuals in the first and second juvenile stages, little or no change in the third and fourth juvenile stages and adolescent stage, and a decrease in the adult stage.

4. Discussion

In an earlier study, acute mortality (48 h) and a direct measure of population growth rate, the instantaneous rate of increase (Stark and Banks, 2001), were reported for diazinon and spinosad as well as several other

Table 1
Stable age distribution (after 65 days) of *D. pulex* after exposure to spinosad and diazinon

Treatment (μg/L)	Stable age distribution (%) in each life stage					
	First juvenile	Second juvenile	Third juvenile	Fourth juvenile	Adolescent	Adult
Spinosad						
Control	20.76	16.45	13.04	10.34	8.19	31.22
2	17.29	14.35	11.91	8.20	8.21	40.04
4	16.47	13.87	11.67	9.83	8.28	39.88
6	15.02	13.08	11.38	9.91	8.63	41.98
8	16.13	14.43	12.91	11.56	10.34	34.63
10	13.82	16.90	22.13	19.67	9.44	18.04
Diazinon						
Control	20.76	16.45	13.04	10.34	8.19	31.22
0.1	21.43	16.84	13.24	10.40	8.18	29.91
0.2	17.90	14.70	12.08	9.93	8.16	37.24
0.3	21.09	16.67	13.17	10.41	8.23	30.43
0.5	21.01	16.60	13.12	10.37	8.20	30.70
0.7	20.41	16.26	12.95	10.31	8.22	31.85
0.8	29.08	20.72	14.76	10.52	7.49	17.43
0.9	29.19	20.96	15.05	10.80	7.76	16.24
1.0	29.08	20.72	14.77	10.52	7.50	17.41
1.5	29.98	22.70	17.19	13.01	9.85	7.27

insecticides. However, in the present study, detailed effects of spinosad on demographic parameters of *D. pulex* were determined through the life table response experiments. In the earlier study, diazinon was found to be 208 times more toxic than spinosad based on acute LC_{50} (Stark and Banks, 2001). However, in the present study, extinction occurred in *D. pulex* after exposure to 2 μg/L diazinon for 2 days and 10 μg/L spinosad for 8 days (Fig. 1). Thus, diazinon was approximately five times more toxic than spinosad based on extinction. A comparison of R_0 values yielded similar results. R_0 was zero after exposure to 2 g/L diazinon and 10 μg/L spinosad. Therefore, based on R_0, diazinon was also five times more toxic than spinosad. Furthermore, recommended field application rates for spinosad are much lower than those for diazinon (Stark and Banks, 2001). Because hazard is a function of susceptibility and exposure (Stark, 2000), spinosad should pose less of a hazard to *D. pulex* than diazinon.

It is astonishing that such large differences exist between acute LC_{50} estimates for spinosad and diazinon (Stark and Banks, 2001) but that these differences are much less when life table parameters are compared. This may be explained by the fact that the birth rate of *D. pulex* exposed to diazinon actually increases over a range of concentrations before finally declining. Thus, even though diazinon is more acutely toxic than spinosad, exposure to this pesticide actually stimulates birth rate. Individuals surviving diazinon exposure produced more offspring than untreated (control) individuals and therefore populations exposed to diazinon are less affected than predicted by the acute LC_{50}.

Demography is being used more frequently to evaluate toxicity (van Straalen and Kammenga, 1998; Forbes and Calow, 1999; Kammenga and Laskowski, 2000). Because demography takes into account all effects (lethal and sublethal) that a toxicant might have on a population and these studies are usually conducted throughout the life span of an organism, a complete measure of effect can be obtained (Stark and Banks, 2000). A comparison of demographic and other endpoints of toxic effect has indicated that demographic toxicological endpoints are superior to other endpoints of effect (Forbes and Calow, 1999). Therefore the demographic approach to the evaluation of toxic effects should be more widely adopted.

5. Conclusion

The effects of two insecticides, spinosad (a new natural insecticide) and diazinon (a commonly used organophosphate), on *D. pulex* were evaluated using a life table approach. Spinosad was five times less toxic than diazinon based on population extinction and net replacement rate. Exposure to both insecticides resulted in lethal and sublethal effects (reduction in the number of offspring per surviving female). However, unlike spinosad, exposure to low concentrations of diazinon actually led to an increase in reproduction. Stable age distributions were affected differently by exposure to each insecticide. Because spinosad is less toxic to *D. pulex* than diazinon and it is applied at lower concentrations it should be less hazardous to this species.

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